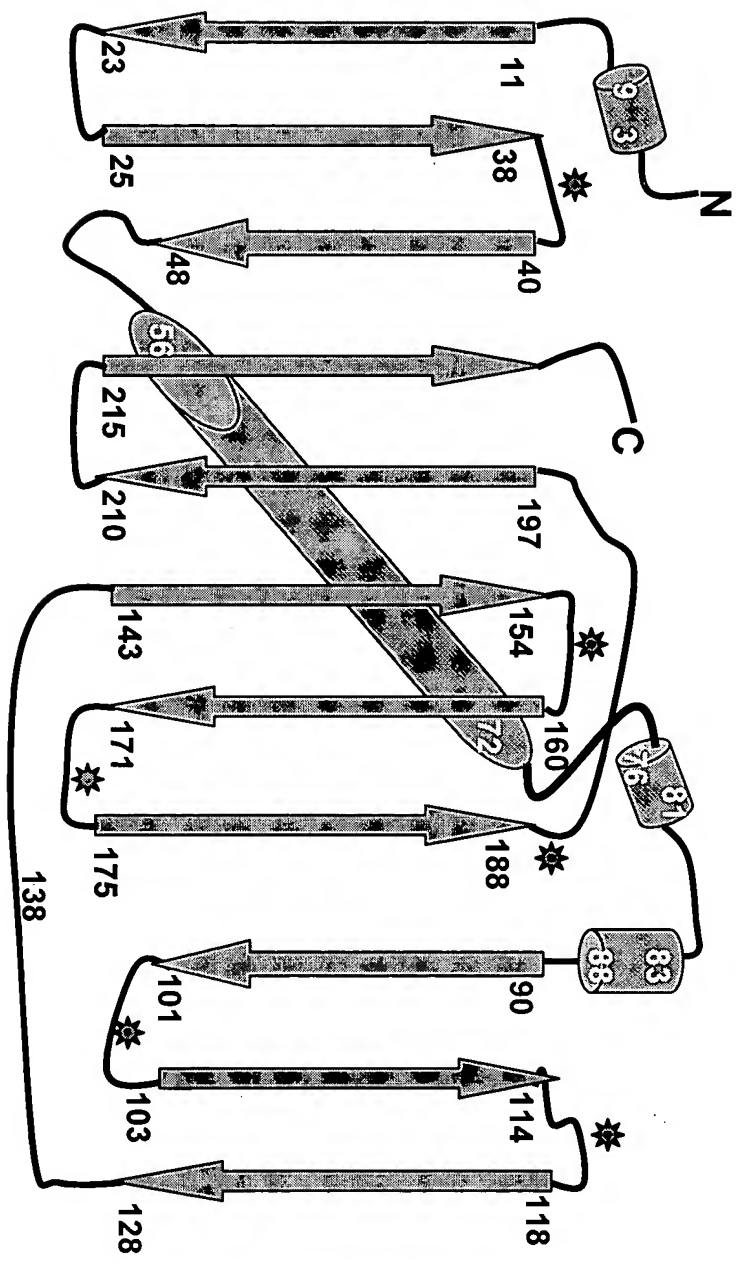


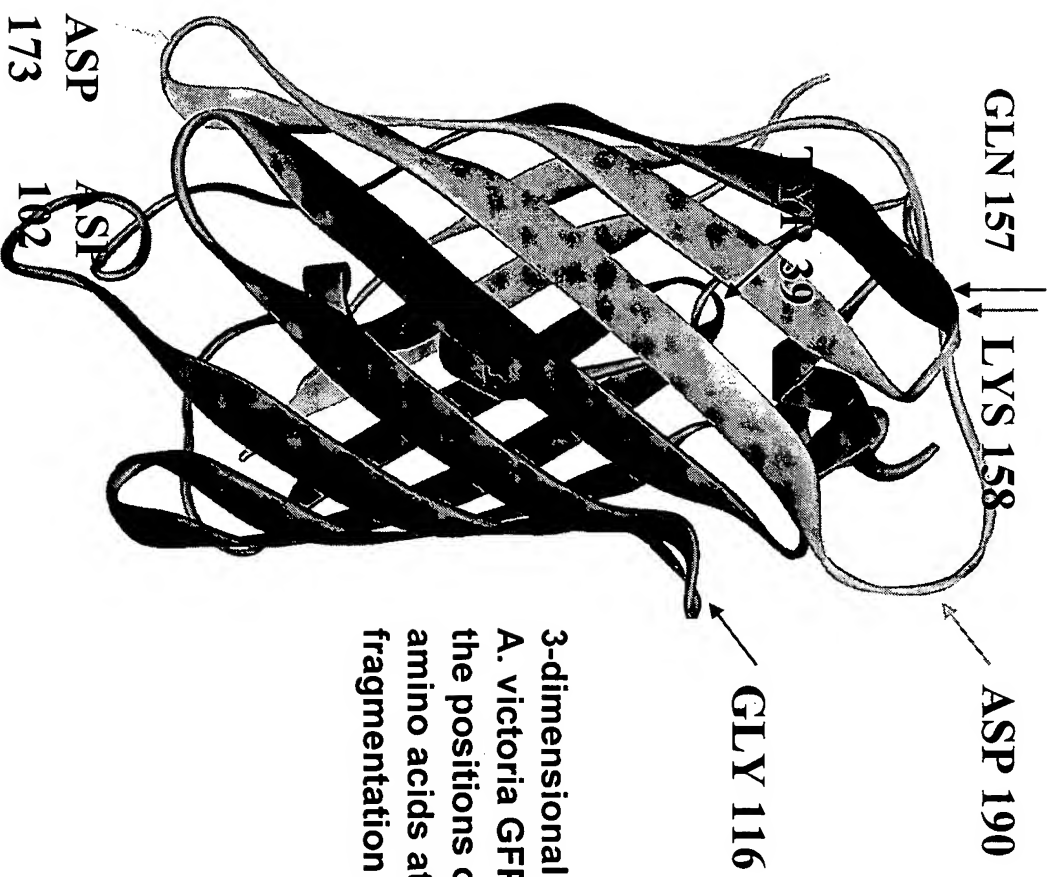
Fig. 1a



Aequorea Victoria Green Fluorescent Protein (GFP)

✱ Loops highlighted by asterisks designate alternative fragmentation sites that are the subject of the invention, comprising amino acid residues 38-40 (region 1); residues 101-103 (region 2); residues 114-118 (region 3); residues 154-160 (region 4); residues 171-175 (region 5); and residues 188-190 (region 6).

Fig. 1b



3-dimensional structure of
A. victoria GFP showing
the positions of specific
amino acids at 6 alternative
fragmentation sites

1. Select a fluorescent protein and its corresponding DNA sequence
2. Create fragments and mutant fragments by:
 - A. Rational design, followed by genetic engineering or oligo-nucleotide synthesis; and/or
 - B. Random or directed fragmentation of full-length DNA molecule
3. Create 5' or 3' fusions of fragments (F1,F2) with genes encoding other proteins (A,B) in expression vectors
4. Co-transfect fragment pair fusions into cells; allow expression of fusion proteins A,B
5. Measure fluorescent signal, e.g. by fluorometry, FACS, or microscopy

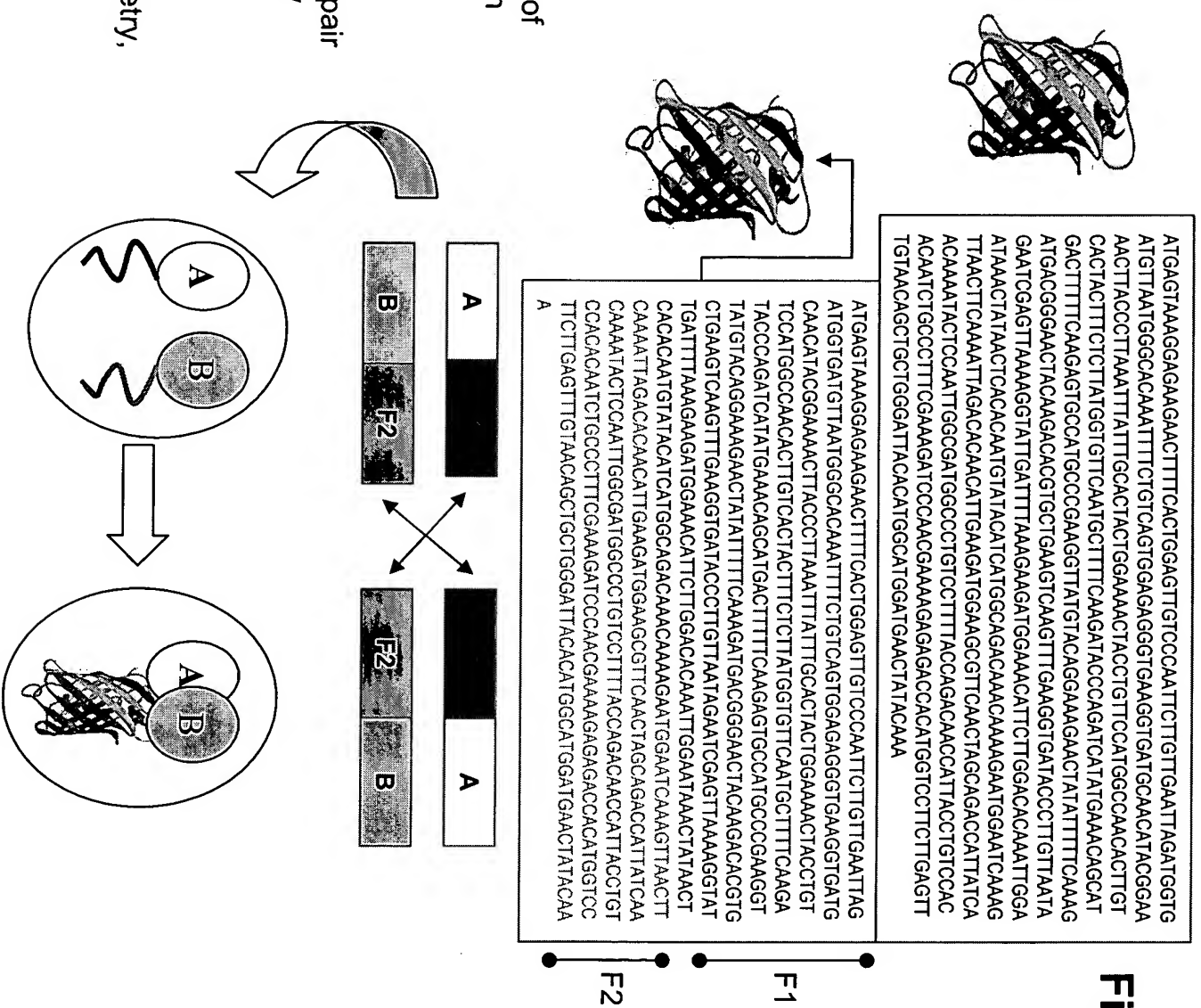
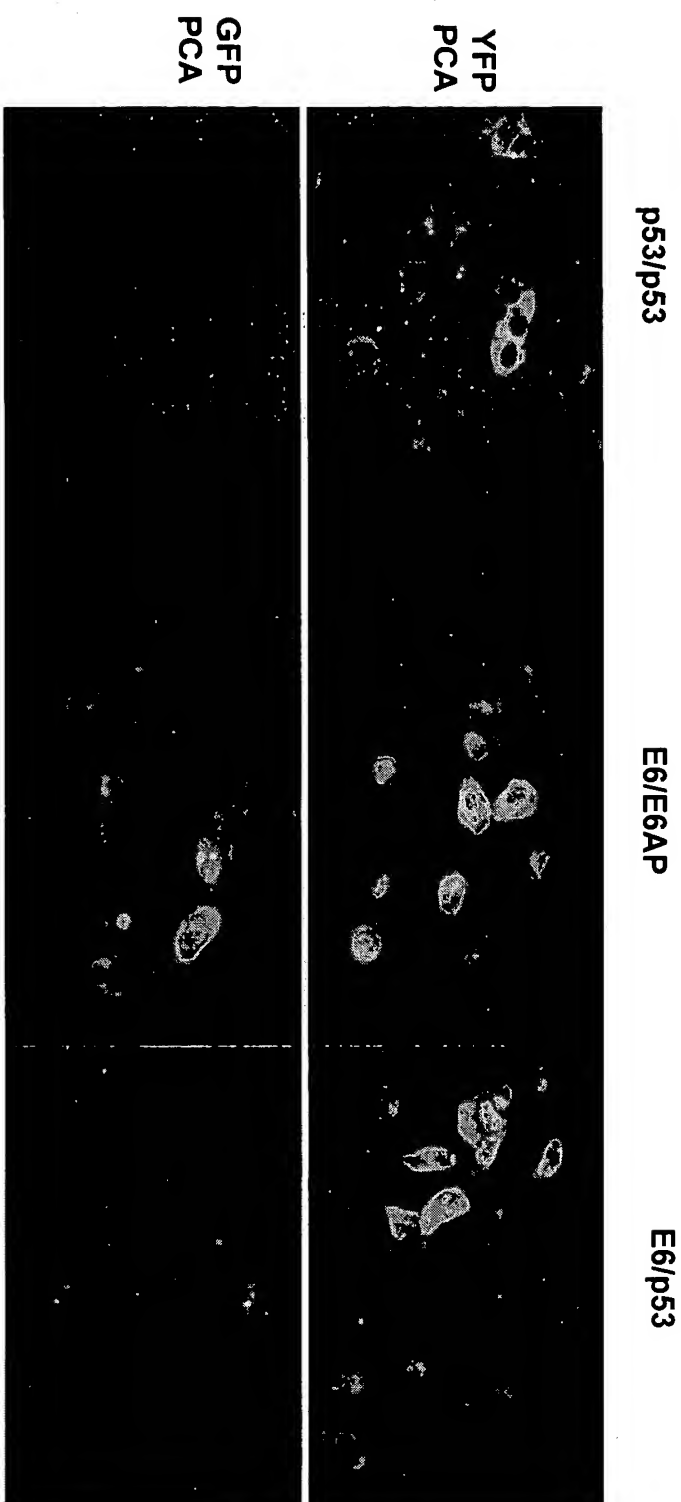


Fig. 2

Fig. 3



24 Hour Expression in HEK293E Cells (1.2 sec exposure)

Fig. 4
Examples of PCAs based on alternative fragmentation sites of YFP

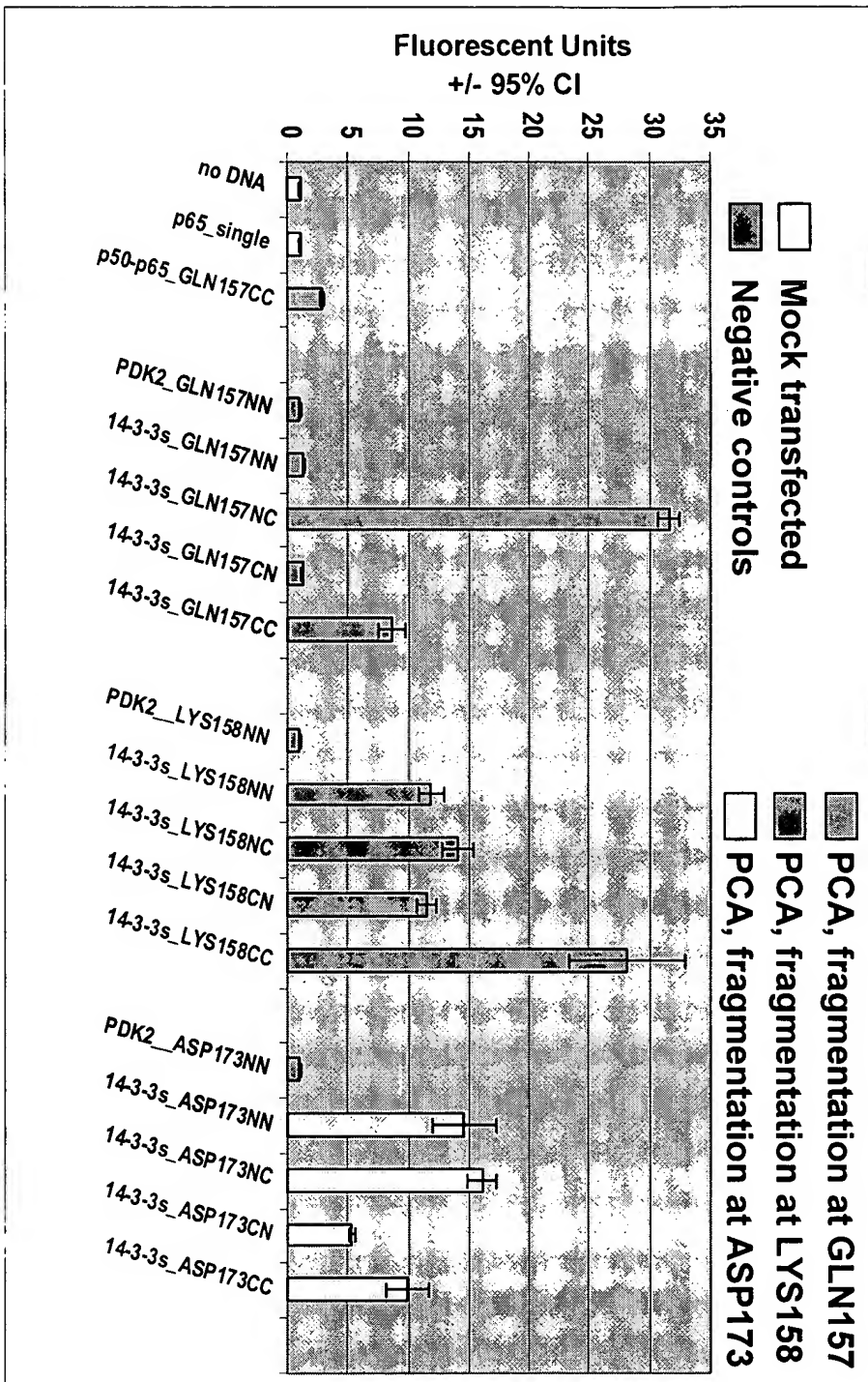
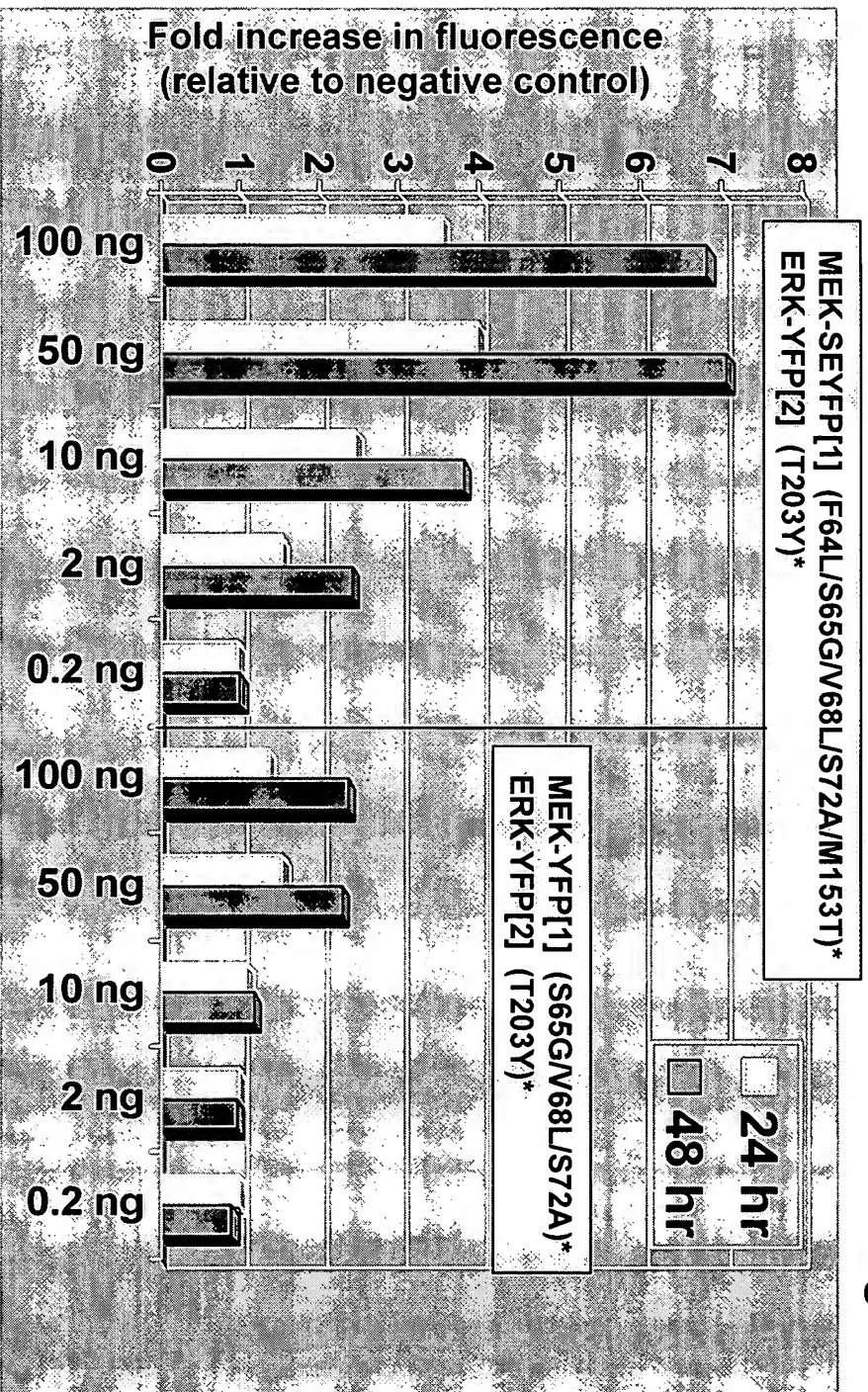


Fig. 5

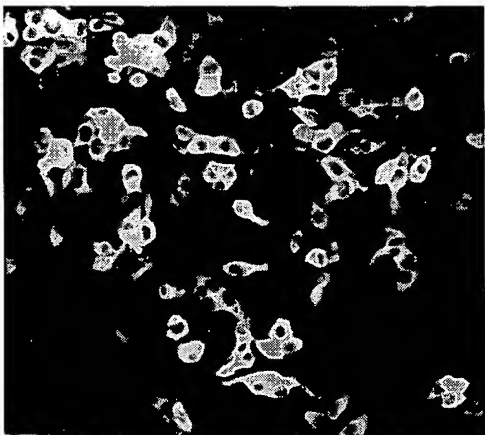


* Note: amino acid changes are designated relative to wild-type GFP

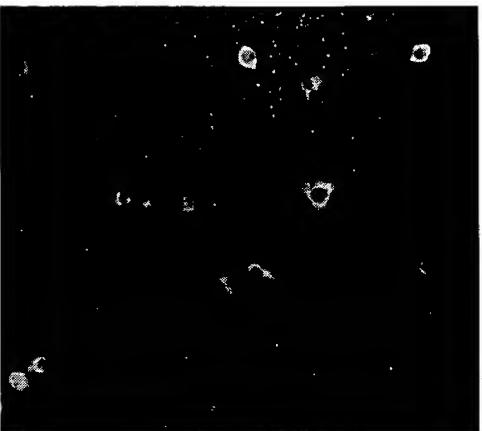
ADOC E7B7IIVAV 1SEB

Fig. 6

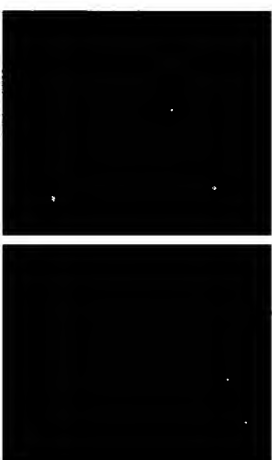
- a. MEK-SEYFP[1] (F64L/S65G/V68L/S72A/M153T)*
ERK-YFP[2] (T203Y)*



- b. MEK-YFP[1] (S65G/V68L/S72A*)
ERK-YFP[2] (T203Y*)



- c. Single fragment control:
Pdk2-YFP[1] (F64L/S65G/V68L/S72A)



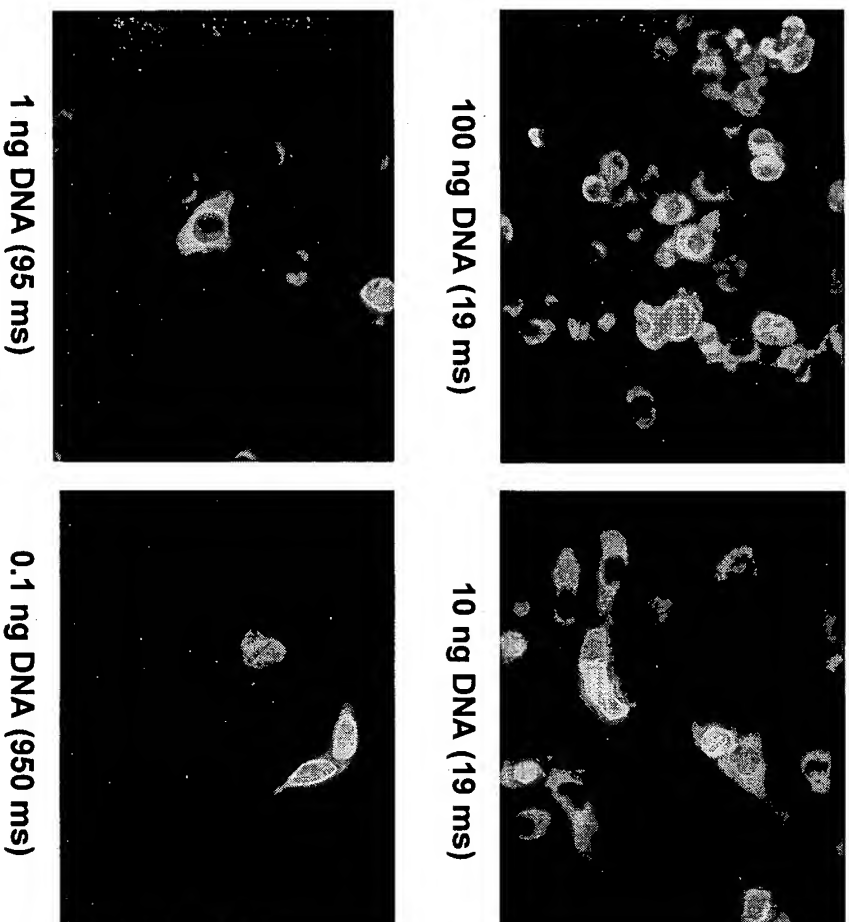
- d. Single fragment control:
Pdk2-YFP[2] (T203Y)



* Note: amino acid changes are designated relative to wild-type GFP

Fig. 7a

An intense fluorescent PCA (IFP PCA) based on mutant fragments of YFP



Results shown are for MEK-IFP[1] + IFP[2]-ERK

IFP[1]: F46L/F64L/S65G/V68L/S72A/M153T

IFP[2]: V163A/S175G/T203Y

Note: amino acid changes are designated relative to wild-type GFP

Fig. 7b.

Enhanced PCA signals based on mutant fragments:

IFP (SEYFP-F46L) versus EYFP

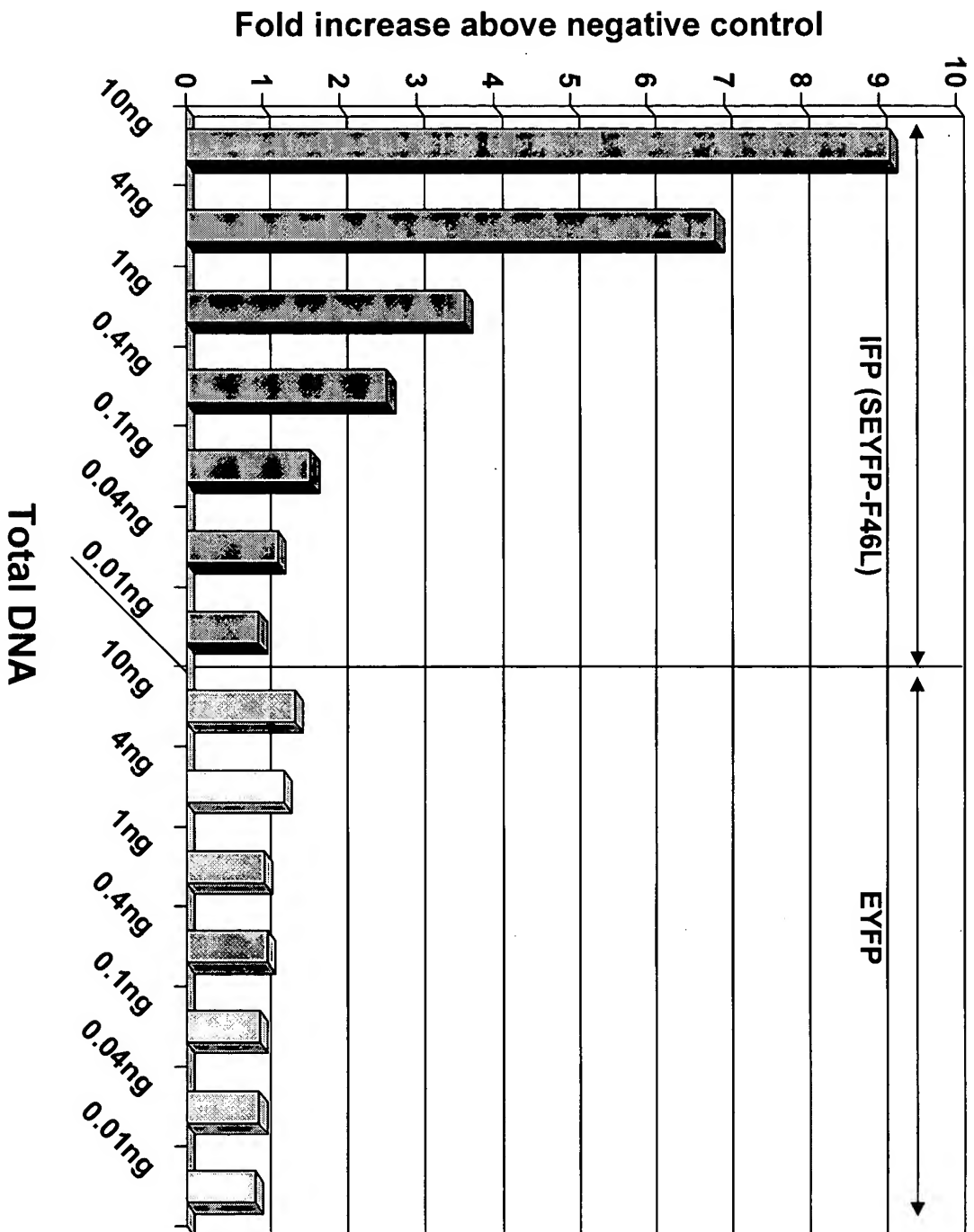


Fig. 8

High-content assays based on IFP PCA:
cytokine-dependent translocation of p65/p50

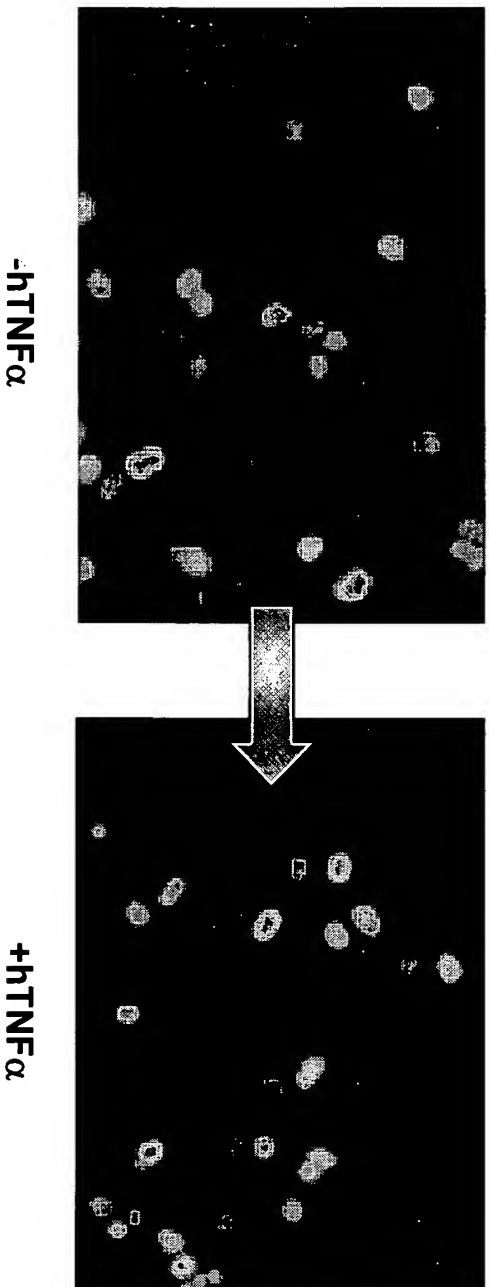


Fig. 9 .
Spectrally shifted (blue) PCAs based on mutant fluorescent protein fragments
(excitation= 436 nm, emission= 480 nm)

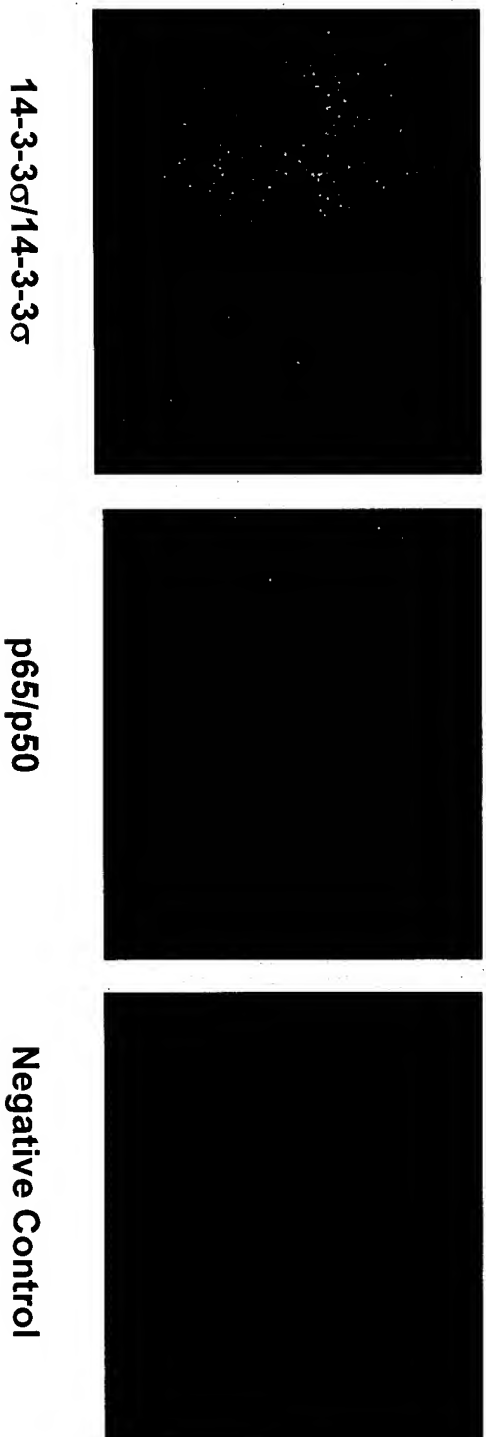


Fig. 10.
Multi-color protein-fragment complementation assays (PCA)

FITC channel

Excitation=460-500 nm

Emission = 505-560 nm